

## PARTHENOGENETIC CLEAVAGE OF THE ARMADILLO OVUM.

H. H. NEWMAN.

### INTRODUCTION.

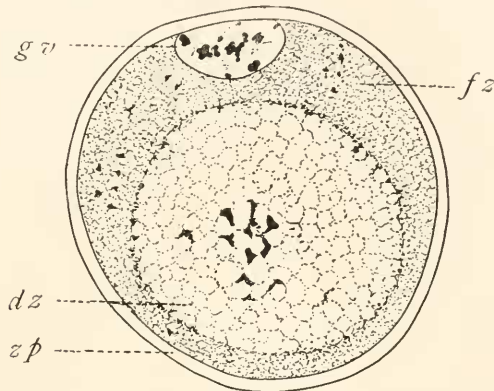
In the year 1909 I began a general study of the ovary of the nine-banded armadillo in search of some explanation of the phenomenon of polyembryony. Among the first of the peculiar features to catch my attention were what appeared to be cleavage stages in certain types of ovarian ova. It was thought that this condition might have some bearing on polyembryony but further study and a review of the literature on kindred topics convinced me that these changes were in no sense to be interpreted as stages in normal development. They must, on the contrary, be interpreted as more or less abnormal and abortive attempts, on the part of ovocytes that have reached maturity but have been denied the normal culmination of their destiny in ovulation and fertilization, to develop embryos in spite of insuperable difficulties. I should hesitate to contribute another chapter to the already voluminous literature on the supposed parthenogenesis of mammalian ova during follicular atresia had I not at my command evidence of a very crucial character, which seems to me to demonstrate beyond controversy a certain amount of real parthenogenesis in a mammal.

The ovaries studied are the same as those that formed the basis of a recent paper on maturation and fertilization in this species (Newman, 1912). Those ovaries, fixed in freshly made Zenker's fluid and stained by Bensley's copper chrome hæmatoxylin process, give the clearest pictures.

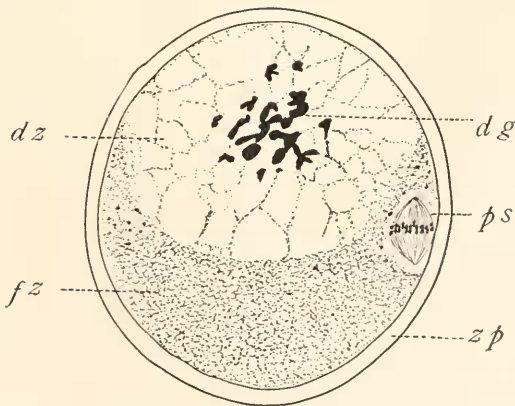
### RÉSUMÉ OF THE EVENTS OF MATURATION LEADING UP TO PARTHENOGENETIC CLEAVAGE.

In order that the changes preceding cleavage may be understood it will be necessary to review the facts dealing with the maturation process which were described in a recent paper (Newman, 1912). The most striking peculiarities of this process

have to do with the reorganization of the cytoplasmic materials of the ovocyte. In this respect, and probably in many others, the armadillo bears a marked resemblance to the marsupials, especially *Dasyurus*, the Australian native cat, whose early develop-



TEXT-FIG. 1. A full-grown ovocyte, showing cytoplasmic organization, etc. Deutoplasmic zone (*dz*), formative zone (*fz*), germinal vesicle (*gv*), zona pelucida (*zp*) ( $\times 410$ ).



TEXT-FIG. 2. A maturing ovocyte, showing the new reversed polarity. The ovocyte is placed with the animal pole upwards. The deutoplasmic zone (*dz*) occupies the animal pole, the formative zone (*fz*) occupies the vegetative pole. The polar spindle (*ps*) lies in a tangential position at the equator of the ovocyte. Deutoplasmic granules (*dg*) lie in the center of the deutoplasmic mass. The zona pelucida (*zp*) is a dense envelope, without radiations.

ment is so ably described by Hill (1910). So close is this resemblance that any description given for one species would apply in almost every detail to the other.

The full grown ovocyte of the armadillo has the structure shown in text-figure 1. The cytoplasm shows two clearly defined regions, a peripheral zone of deeply staining homogeneous protoplasm, the *formative zone* (*f.z.*), and a central lightly staining, spherical mass of coarsely reticular or alveolar material, the deutoplasmic zone (*d.z.*), in which occur irregular masses of coarse and deeply staining granules (*d.g.*), the supposed equivalent of the yolk or deutoplasmic granules of the ova of marsupials. The germinal vesicle (*g.v.*) is flattened against the dense zona pelucida (*z.p.*) at the animal pole and in it the chromatin is seen as tetrads or in other forms characteristic of the prophase of maturation. Such an ovum has the appearance of being centroleithal. Coincident with the formation of the first maturation spindle a radical rearrangement of the two cytoplasmic zones occurs. The deutoplasmic sphere pushes its way to the surface at the animal pole and crowds the formative material to the vegetative pole where it assumes the form of a cap, thick at the pole and thinning out to a feather edge at the equator (see text-figure 2). The polar spindle, in a stable metaphase (*p.s.*), occupies a position as near the animal pole as possible without leaving the formative material and has its axis tangential to the periphery of the ovocyte though parallel with that of the main axis of the cell. This spindle is evidently insulated from the surrounding formative protoplasm by a capsule of non-staining material, and has no astral rays.

A very large number of ovocytes were found in exactly this condition and it seems certain that the progressive changes normally come to a standstill at this point. The next step in normal development is ovulation, the stimulus of which initiates the completion of the maturation process. Such resting ovocytes occur in large follicles whose granulosa cells are intact and whose follicular fluid is homogeneous, abundant, and stains deeply with hæmatoxylin and allied stains. In any normal ovary during the period of œstus there are many ovocytes of this sort, all in practically the same condition and equally ready for ovulation. Probably such mechanical factors as size of follicle or nearness to the periphery determine which follicle shall rupture first. Fertilization of this one egg seals the fate of all the remaining

ovocytes of the same generation in that they can never go through the process of ovulation and therefore cannot develop normally. A very small percentage of these ovocytes continue the maturation process to the extent of completing the first maturation division; a still smaller proportion (only three out of hundreds of cases examined) complete the second maturation; while the remainder, over 90 per cent., are struck by the processes of follicular atresia and either go into early cytolysis or enter upon a period of parthenogenesis. It is with the latter contingency that the present paper deals.

#### ABSTRICTION OF THE DEUTOPLASMIC MATERIAL AND ITS SUBSEQUENT FATE.

The condition of equilibrium, just described and illustrated in text-figure 2, is normally disturbed only by ovulation, but, in the case of ovocytes whose normal history has been cut short by the fertilization of an ovum and the formation of a corpus luteum, the equilibrium may also be upset by the marked changes in the chemical environment incident to follicular atresia. The follicle in which atresia has set in rapidly decreases in size through the loss of follicular fluid, but, even before the amount of fluid has materially diminished, its chemical composition is clearly altered, for it no longer stains deeply with the ordinary cytological stains but remains pale gray under hæmatoxylin treatment. Just what the nature of the change is I am unable to determine, but there can be no question as to its radical character. As atresia continues, the lumen of the follicle gradually disappears, partly through the loss of fluid and partly through the ingress of cells from the disintegrating granulosa layer of the follicle. With this radical alteration of chemical environment the ovocytes show marked changes, whose significance it is our problem to determine.

The first act of the ovocyte entering upon parthenogenetic development is the abstriction of the deutoplasmic mass from the formative protoplasm. That this is a practically universal phenomenon can scarcely be doubted for there are in the present material literally hundreds of instances of it, and one can with confidence look for some stage of the process in every follicle



that has reached the mid-period of atresia. Abstriction of the deutoplasm is essentially an act of rejuvenation on the part of a dying cell, in that the still living protoplasm is freed from the burden of the inert by-products of metabolism. The exact mechanics involved in this purification process can scarcely be determined from the fixed material, but appearances seem to warrant the conjecture that the very fluid deutoplasmic material escapes from the cell membrane by the rupture of the latter and that, with the subsequent closure of the ruptured membrane, the deutoplasm becomes strictly extra-cellular and without effect, except in a secondary mechanical way, upon the living protoplasm of the germ cell. Freed from its burden the renewed egg-cell rounds up into an approximately spherical form and apparently floats in the deutoplasmic fluid. It is of considerable interest to note that a similar abstriction of deutoplasmic material occurs as the initial step of normal development in *Dasyurus*. Hill shows that this discarded material plays no further part in development but merely occupies any available space within the persistent zona pelucida that is not occupied by the blastomeres. Finally it comes to lie in the cleavage cavity of the blastula and is probably gradually absorbed. In view of the striking similarity in the early history of the deutoplasm in the two species it is difficult to avoid the conclusion that the process, as described for these ovarian ova of the armadillo during atresia, is a close approximation of the first step in normal development, about which we know practically nothing at present.

The deutoplasmic fluid, laden with its masses of deutoplasmic granules, usually gives the appearance of a multicellular body, but I am inclined to consider this condition as largely an artefact. It is not improbable that the strong fixing fluids used coagulate this somewhat viscous material in the form of many small rounded masses. In a few cases, as in Figs. 2 and 4, the deutoplasm is only slightly broken, but in the majority of instances we find advanced fragmentation, as in Figs. 1, 3, 5, etc. Whether the deutoplasm occurs in one or many fragments the deutoplasmic granules occupy a central position in the various pieces giving each fragment the illusory appearance of a nucleated cell. These are not however to be interpreted as in any sense true

cells. At best they may be designated as *cytoids*. Other writers who have studied similar conditions in mammals have referred to the existence of multicellular conditions in which some cells possess nuclei and others do not. I suspect that, were the truth known, these fragments without nuclei would turn out to be of deutoplasmic origin. Very commonly these cytoids in our material form a pseudo-epithelial layer surrounding the true cells, as in Figs. 3 and 5, or less distinctly in Fig. 1. In other cases they are distributed at random among the cellular products of cleavage (Figs. 7-11) and serve only to give a false appearance of cellular multiplicity and to confuse and obscure the pictures of cleavage.

#### THE REESTABLISHMENT OF NORMAL INTERRELATIONS BETWEEN THE NUCLEUS AND THE CYTOPLASM AND THE RESULTANT FORMATION OF CLEAVAGE SPINDLES.

The egg, much reduced in size but with a renovated cytoplasm, is now the seat of a renewal of nucleo-cytoplasmic exchanges, as evidenced by the appearance of an extensive system of astral rays at the two poles of the spindle. It will be recalled that the nucleus during the period prior to ovulation was a naked spindle, enclosed in an insulating capsule and entirely devoid of astral radiations. Such a spindle is evidently an isolated system and lies inert in the cytoplasm. The renovated cytoplasm of the egg from which the deutoplasm has been extruded now bears a different chemical relation to the nucleus and the change is shown in the disappearance of the capsule about the nucleus and in the appearance of typical astral radiations. That a renewal of metabolic relations between nucleus and cytoplasm is one of the most essential facts of normal fertilization has been recently maintained by Lillie (1911) on the basis of his studies of fertilization in *Nereis*, and there is reason to believe that this is the physiological explanation of parthenogenesis wherever the latter is found, whether normal or experimental. In this case it is my conviction that the sudden change in the chemical character of the cytoplasm restores the basis of life and growth to the egg and that cleavage is the natural consequence. In such cases as that shown in Fig. 1 the nucleus has returned to a

resting state and has every appearance of undergoing healthy changes which should culminate in cell division by mitosis. It is impossible to state positively that such a nucleus has regressed from the spindle condition shown in text-figure 2, but that such is the case seems at present to be a reasonable conjecture. In eggs of this sort there is a characteristic zone of activity in the cytoplasm at a short distance from the nuclear membrane which may indicate active interchanges between nucleus and cytoplasm. The chromatin is in the form of a series of beaded threads resembling a spireme and therefore may be interpreted as in a very early prophase of the first cleavage. Soon the chromosomes begin to condense and the typical appearances of later prophases pass over into unmistakable mitotic figures like that shown in Fig. 4, which can be interpreted in no other way than as cleavage spindles. The so-called cleavage mitoses of other writers on this subject have been interpreted by opponents as belated maturation spindles which have been displaced and distorted by the abnormal conditions of atresia; but such an interpretation of the figures here described would seem very far-fetched in view of the fact that these spindles are so radically different from the naked, insulated spindles of maturation. I have never seen a vestige of astral radiations in a polar spindle, while the radiations in a number of such cases as that shown in Fig. 4 are as obvious and as extensive as those which I have observed in the cleaving eggs of annelids and other favorable material, and far clearer than similar appearances in fish eggs or other less favorable material. The spindles are frequently abnormal in form, being not uncommonly tri- or multipolar; and sometimes the poles are ill defined, as in Fig. 4, but the radiations are always very distinct. When the figure is multipolar the chromatin distribution is very irregular and nests of nuclei (like those shown in Fig. 12) are produced without any division of the cytoplasm. It is very common to find such multinucleate but unicellular eggs and I am inclined to attribute their existence to such irregular mitoses as that just mentioned. Certain considerations lead me to venture a conjecture as to the mode of origin of these multipolar spindles. It is not uncommon to find in oocytes of the type shown in text-figure 2 various steps

in the anaphases of the first maturation division, which might readily result in the formation of two nuclei without the extrusion of a polar-body. Conditions like those shown in Figs. 2 and 3 are not nearly so abundant as those like Fig. 1, but they are about as common as are multipolar spindles and might readily result in the latter type of abnormality. Eggs with such paired nuclei have the general appearance of fertilization stages but cannot be interpreted as such, unless the reunion of the polar nucleus with its sister, the nucleus of the secondary ovocyte, could be considered a sort of fertilization process. It is not difficult, however, to see how double nuclei of this sort could form just such multipolar spindles as that shown in Figs. 13 and 14. It will be readily noted from the figures that these abnormal spindles have a very much larger number of chromosomes than sixteen, the number seen in all clear maturation figures. This in itself militates against the interpretation of these phenomena as maturation processes, and is in accord with the conjecture that the figures may be the result of the coöperation of two nuclei derived from a maturation division.

The bipolar spindles, such as that shown in Fig. 4 (see also Figs. 15 and 16), also contain more than the haploid number of chromosomes and cannot on that account be maturation figures.

#### EARLY CLEAVAGE STAGES RESULTING IN THE FORMATION OF A FEW BLASTOMERES.

In the course of these studies several very pretty two-cell stages have been encountered of which those shown in Figs. 5 and 6 are typical. The stage shown in Fig. 5 is a perfect two-cell stage in which both nuclei are in the resting phase. There are no other nucleated bodies within the zona pelucida, but a pseudo-epithelium of deutoplasmic cytoids forms a capsule about the two blastomeres. A clear case of a two-cell stage in which the second cleavage is well under way is shown in Fig. 6. The cleavage spindles are cut transversely in both cells, one of which shows an early and the other a later anaphase.

After the second cleavage the subsequent divisions are less normal and the picture of blastomeric regularity is confused. The spindles lose distinctness, as a rule, and the chromatin

begins to break down, giving appearances like those shown in Figs. 7 and 8, which are two sections through the same egg. Another example of the same condition is shown in Figs. 9 and 10, also taken from one egg. In Fig. 7 can be seen one cell with a fragmenting nucleus, a degenerating nucleus in another cell, and an incomplete mitotic figure with only a few small chromosomes. In Fig. 8 occur three cells all of which show abnormal, though unmistakable, mitotic figures. Such an egg might be considered as a six-cell stage, with a prospect of reaching a ten-cell condition. There is every evidence, however, of approaching death and disintegration in such cases and one would not be inclined to look for much further developmental progress in such unpromising material. In the egg shown in Figs. 9 and 10 there are several imperfect spindles and two small but healthy nuclei. One might be somewhat more optimistic about the ultimate fate of cases of this sort, but, since these cases show about the maximum of development in the present material, such optimism is scarcely warranted.

Fig. 11 is introduced especially to show an unusually fine mitotic figure in one of several cells in an egg in which three other cells show more or less normal nuclei. This egg occurred in a follicle in a very advanced stage of atresia and it is rather surprising to find so pronounced a spark of life in a structure so nearly dead.

In all of the cases cited and in nearly all in which similar phenomena were observed the zona pelucida was dense and quite intact, hence there can have been no invasions of stroma or follicular cells. So all nuclei found must be products of the division of the original germinal vesicle. In more advanced atresia, however, the zona begins to open up cracks which admit hordes of stroma cells and leucocytes that feed upon the disintegrating egg material and form cell masses that have been interpreted by some authors as the products of embryonic development. In such cases the observer might be inclined to interpret such synthetic structures, which frequently have an epithelial structure, as tissues, equivalent to those formed in normal embryonic development. Leo Loeb ('11 and '12) in recent papers interprets as placental tissues certain structures



found under similar conditions in the guinea-pig ovary. Not having seen his preparations, but simply judging by his microphotographic illustrations, I am inclined to think that, since he gets no intermediate or advanced cleavage stages, the cell complexes he discusses are open to the interpretation just given for similar phenomena in the armadillo, about whose synthetic origin there appears to be no doubt. Parthenogenetic development in all probability goes no farther in the armadillo than the stages illustrated in the figures. That true parthenogenesis begins and proceeds for a few steps seems assured, but it seems highly improbable, *a priori*, that development could long continue in an environment so unfavorable as that afforded by follicles in which atresia has made such progress as we have seen.

#### DISCUSSION OF THE LITERATURE ON PARTHENOGENESIS IN MAMMALS.

In 1900 Bonnet in an able paper entitled "Giebt es bei Wirbeltieren Parthenogenesis?" reviewed all the literature dealing with parthenogenesis in vertebrates and gave much space to the question of parthenogenesis in mammals. He concluded that there was no incontrovertible evidence of this mode of development in any of the contributions dealing with the changes described as occurring in ovarian ova during follicular atresia. A considerable number of authors had described ovarian ova containing centrally situated mitotic figures which they had interpreted variously as cleavage spindles or as belated maturation figures. Multicellular masses within zona pelucida were described, in which some cells contained nuclei and others were without nuclei. Some writers considered this condition as a result of a degenerative fragmentation of nucleus and cytoplasm, and others were convinced that the cell mass was the product of parthenogenetic cleavage. Another class of writers called attention to the existence of various kinds of more or less complex ovarian dermoids, epithelioma and other teratoma, which by some were interpreted as the end product of the parthenogenetic development of ovarian ova. Judicially examining all of the evidence before him Bonnet concludes that all of the mitotic figures seen in ovarian ova are to be considered not as



cleavage mitoses but as more or less abnormal maturation figures, that the so-called multicellular embryos are degenerative products, and that teratoma and other kindred phenomena must be explained in some other way than as the products of parthenogenetic cleavage of ovarian ova. This conclusion is, I believe, fully justified by the evidence then available.

Since this review and judgment of Bonnet several authors have reopened the question but have failed to agree. Spuler (1900) insists that the figures which he finds located in the center of ovarian ovocytes, after the extrusion of one polar body, are cleavage spindles, because normally ovulation occurs before this stage and the ovocytes do not develop thus far unless fertilized. He places especial emphasis on finding one ovum in which a centrally located spindle occurred in an egg that had two polar bodies. This, however, is probably a case similar to that described by the present writer (Newman, '12) as due to a precocious division of the first polar body, the spindle being merely the second maturation spindle. Spuler fails to add any material strength to the affirmative side of the question reviewed by Bonnet.

Van der Strict (1901) working with the bat ovary also took the affirmative side of the question as the result of his discovery that ovocytes of the second order occasionally divided mitotically into two cells of approximately equal size. This division could not, he thought, be considered as an anomalous polar body formation, nor as a mitotic division due to degeneration, nor as fragmentation, but only as the beginning of parthenogenetic division. I am inclined to think that Van der Strict was dealing with phenomena closely akin to those described in the present paper for the armadillo, but he failed to make his evidence especially convincing.

L. Loeb (1901) takes a position similar to that assumed by Spuler and Van der Strict and goes a step further in that he holds that the subsequent fragmentation of the egg material into nucleated and enucleated pieces is a progressive phenomenon akin to the parthenogenetic development of the egg of *Chaetopterus* which, as Lillie has shown, develops into a larva of considerable complexity without any nuclear cleavage. Loeb figures

one multicellular mass in which a single cell is dividing mitotically, but the spindle is of the size and character of a polar spindle and lacks astral rays. Under the circumstances such a spindle would probably be capable of interpretation as the first polar spindle of an egg that had undergone degenerative fragmentation.

Rubaschkin (1906), as the result of his studies of the guinea-pig ovary, takes a decidedly negative stand, maintaining that all mitotic figures occurring in ovarian ova during follicular atresia are more or less distorted or otherwise abnormal maturation mitoses, and in no sense the mitoses of embryonic cleavage. Multinuclear and apparent multicellular conditions are interpreted by him as the result of degenerative changes brought about by the conditions incident to follicular atresia.

Athias (1908 and 1909) once more reviews the whole situation and presents further data derived from the study of a number of species of mammals. After summing up the evidence pro and con he finds himself unable to reach a definite conclusion though leaning decidedly to the negative point of view. Pending an extensive program of investigation on the subject he prefers to reserve judgment.

My own investigations herewith presented were begun, as stated, in 1909 and, as the reader will have discovered, support the view that a limited amount of parthenogenetic cleavage occurs but that development proceeds no farther than two or three cell divisions.

#### SUMMARY.

The evidence derived from a study of large numbers of armadillo ovaries demonstrates, I believe, that parthenogenetic cleavage takes place in atretic follicles of this species of mammal. Whether cleavage is preceded by maturation is not clear, but I am inclined to believe that no polar bodies are extruded in those ovocytes that are destined to undergo cleavage.

There is strong reason to believe that the abstriction of the deutoplasmic material in these ovarian ova is equivalent to one of the steps in normal development, for a similar process occurs in the development of several marsupials, the ovogenesis of which shows the same history of deutoplasm formation and reorganization as that seen in the armadillo.

There is no evidence that cleavage proceeds beyond the eight-cell stage, and even at that period there are many signs of advancing degenerative processes. It seems certain, therefore, that, in the armadillo at least, no complex tissue masses such as ovarian teratoma or epitheliomata result from a continuation of the process of parthenogenetic cleavage.

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HULL ZOÖLOGICAL LABORATORY,

UNIVERSITY OF CHICAGO,

April 8, 1913.

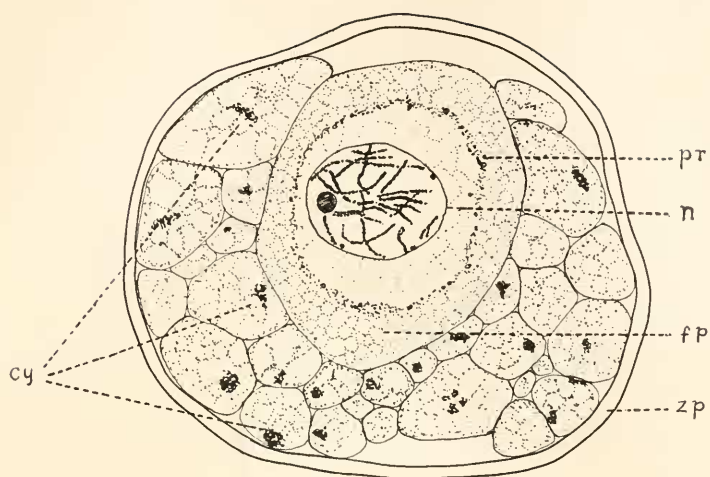


## EXPLANATION OF PLATES.

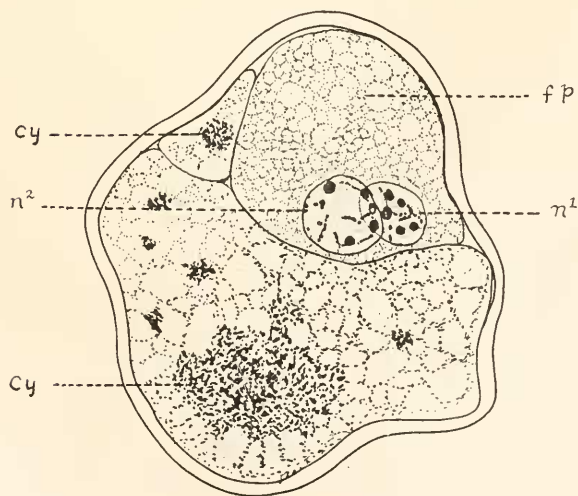
## PLATE I.

FIG. 1. Egg with large resting nucleus, showing resumption of chemical interaction between nucleus and cytoplasm preparatory to the formation of the first cleavage spindle. The deutoplasm has been extruded and has been fixed in the form of numerous cytoids (*cy*) that almost surround the egg. The dense zona peludica (*z.p.*) encloses both egg and cytoids. The egg shows a zone of unmodified formative protoplasm (*f.p.*) and a precipitation ring (*p.r.*), where the changes between nucleus and cytoplasm are most active. Note that each cytoid has a centrally located, deeply staining, mass of granules, which are the deutoplasmic granules of earlier stages and are not to be interpreted as degenerating nuclei. ( $\times 800$ .)

FIG. 2. Egg in which the deutoplasm has just been abstricted and has not yet undergone fragmentation to any marked extent. Two nuclei ( $n^1$  and  $n^2$ ) lie side by side giving the appearance of a fertilization stage. It is probable that these are the product of the first maturation division, completed without the extrusion of a polar body. This could readily occur in cases where the maturation spindle occupies a central position. These two nuclei would doubtless form a multipolar spindle like that shown in Figs. 13 and 14 and would probably result in the formation of nests of nuclei, as in Fig. 12. Other labelling as in Fig. 1. ( $\times 800$ .)



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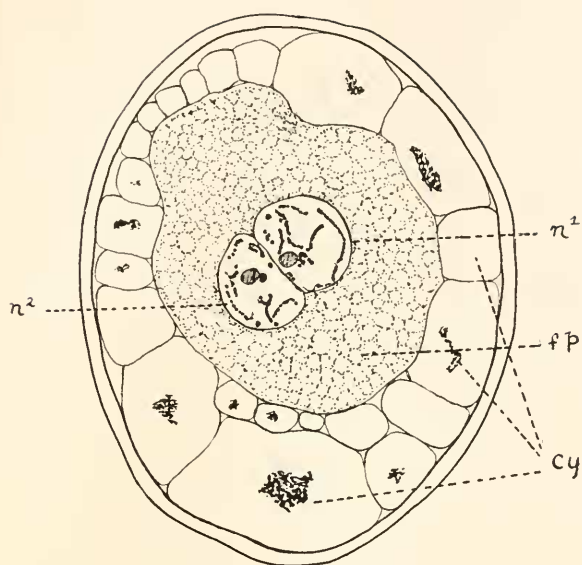




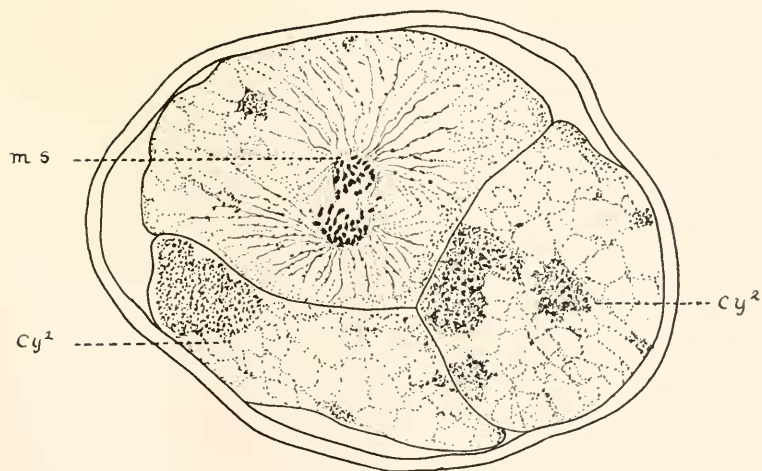
## PLATE II.

FIG. 3. Egg with pseudo-epithelium of cytoids and with binucleated egg cell in the center. The explanations given for Figs. 1 and 2 will apply here. ( $\times 800$ .)

FIG. 4. Egg showing mitotic spindle (*m.s.*) with well-defined polar radiations running from nucleus throughout the cytoplasm. The rather blunt-ended spindle shows mitosis in an anaphase and there are many more than the reduced number of chromosomes, which one invariably finds in maturation spindles. Such a spindle cannot be other than a cleavage spindle. There are only two large cytoids (*cy*<sup>1</sup> and *cy*<sup>2</sup>). ( $\times 800$ .)



3



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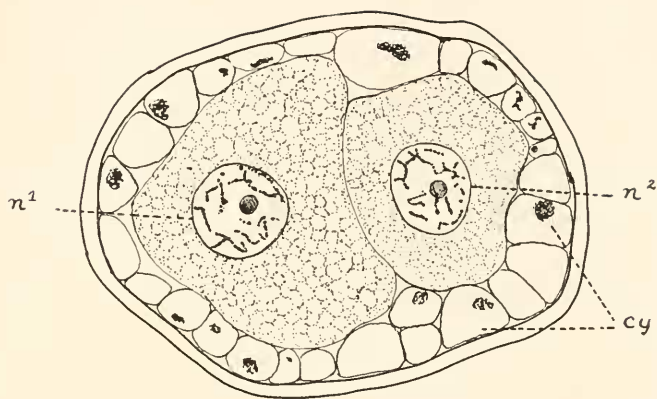




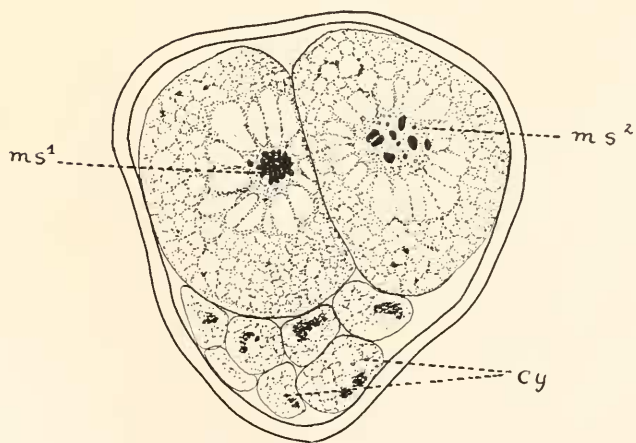
## PLATE III.

FIG. 5. A two-cell stage of cleavage. The two cells are of somewhat unequal size, but the nuclei ( $n^1$  and  $n^2$ ) are both in good condition and in the resting stage. A pseudo-epithelium of deutoplasmic cytoids surrounds the blastomeres. ( $\times 800$ .)

FIG. 6. A two-cell stage passing into the four-cell condition. Two mitotic spindles are cut transversely to the axis. The spindle on the right ( $m.s^2$ ) is cut near the equatorial plate and shows only a few of the chromosomes. Other chromosomes are scattered through several sections. The spindle on the left ( $m.s^1$ ) is in a late anaphase and has two groups of chromosomes, one at each end of the spindle. The present section cuts through only one of these chromosome groups. Large numbers of cytoids occur in other sections. ( $\times 800$ .)



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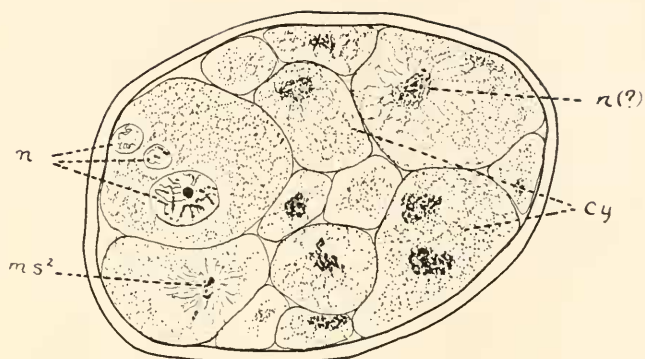
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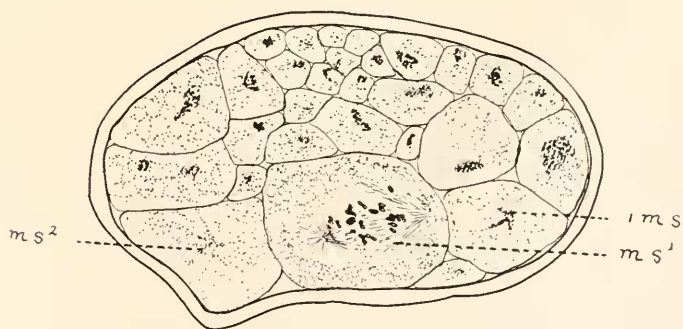


## PLATE IV.

FIGS. 7 and 8. Two sections through the same egg, showing one clearly defined mitotic spindle ( $m.s^1$ ), a cell containing three nuclei in the resting stage, one cell with what appears to be a degenerating nucleus ( $n?$ ), and one cell, shown in both sections, with an imperfect mitotic spindle and only a few chromosomes ( $m.s^2$ ). Cytoids of all sizes are mixed in with the blastomeres. Such an egg seems to be in about a four-cell stage, but will probably not go much further. ( $\times 800$ .)



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